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Effect of sport stress on lymphocyte transformation and antibody formation

J. ESKOLA, O. RUUSKANEN, E. SOPPI, M. K. VILJANEN, M. JÄRVINEN, H. TOIVONEN & K. KOUVALAINEN *Departments of Medical Microbiology, Pediatrics and Anatomy, and Sport Research Unit, University of Turku, Turku, and Department of Pediatrics, University of Oulu, Oulu, Finland*

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SUMMARY

The effect of a heavy (marathon, 2·5 hr) and moderate (35 min of running) sport stress on the number and function of lymphocytes, and on the plasma cortisol and leucocyte levels was investigated. Marathon running had a profound effect on the lymphocytes. Though the total number of lymphocytes did not change, their responsiveness to PHA and Con A, and especially to PPD, was clearly depressed. The suppression of lymphocyte transformation was transient, the recovery occurring in 24 hr. The marathon running had no effect on antibody-forming capacity when the antigen was given immediately after the performance of the marathon, i.e. at the time when the response of lymphocytes to PHA, Con A and PPD stimulation was impaired. A clear-cut granulocytosis and elevation of plasma cortisol was seen in all the marathon runners. The 35 min of running also resulted in granulocytosis and an increase of plasma cortisol, but it did not cause any impairment of the lymphocyte function.

INTRODUCTION

Immune responses in different animal species are depressed by various types of stress. The suppressive influence of a short-term stress on the antibody production has been firmly established by using isolated splenic lymphocytes in mice (Gisler, 1974; Monjan & Collector, 1977) and in rats (Solomon, 1969). The degree of inhibition has been found to be related to the level of plasma 17-hydroxycorticosteroids (Gisler & Schenkel-Hulliger, 1971).

The effect of stress on the cell-mediated immunity is not so well-defined. There are, however, many reports showing that corticosteroids administered both *in vitro* (Heilman, Gambrill & Leichner, 1973; Blomgren, 1974) and *in vivo* (Claman, 1972; Fauci & Dale, 1974; Yu *et al.*, 1974; Balow, Hurley & Fauci, 1975; Clarke *et al.*, 1977) suppress the response of lymphocytes to stimulation by mitogens and antigens. In addition, corticosteroids induce a decrease in the absolute numbers of circulating B and T lymphocytes, but the T-cell lymphopenia is more pronounced (Fauci & Dale, 1974; Yu *et al.*, 1974; Clarke *et al.*, 1977). On the other hand, a short exercise induces a transient lymphocytosis with a predominant effect on the B lymphocytes (Steel, Evans & Smith, 1974; Hedfors, Holm & Öhnell, 1976; Yu, Clements & Pearson, 1977).

In the present work we have studied the effect of a marathon and 7 km of running on the plasma cortisol, total leucocyte and lymphocyte counts, and on the lymphocyte transformation induced by mitogens and purified protein derivative of tuberculin (PPD). In addition, antibody formation capacity was investigated after marathon running.

Correspondence: Dr Jussi Eskola, Department of Medical Microbiology, University of Turku, SF-20520 Turku 52, Finland.

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MATERIALS AND METHODS

Experimental protocol and subjects. In the first part of the study, eight healthy male volunteers (25–50 years of age) served as test subjects. Four of them, competing athletes, regularly exercising long-distance running, ran a complete marathon (42·195 km) in an average time of 2·5 hr and the other four, exercising regularly shorter (5–10 km) runnings, ran 7 km in 35 min. Blood samples were collected 30 min before the run and 30 min and 3 hr after the completion of the run. To evaluate the effect of running, plasma cortisol, total leucocyte and lymphocyte counts and the responses of peripheral blood lymphocytes to phytohaemagglutinin (PHA), concanavalin A (Con A) and PPD were assessed.

When a suppressive effect of the marathon running on the lymphocyte transformation was found, eight highly conditioned long-distance runners (25–30 years of age) were examined in the second part of the study. Blood samples were obtained from these national top-level athletes 30 min before and after the marathon and, in order to study the recovery of the depression of the lymphocyte transformation, on the next day at the same time of day as the first sample. The same parameters as mentioned above were measured in these runners. In addition, they were immunized with 0·5 ml of tetanus toxoid (Orion, Helsinki, Finland) after the second blood sample, i.e. at the time when the responsiveness of the lymphocytes to mitogen and PPD stimulation was depressed. The subjects had received a former tetanus toxoid vaccination within the last 10 years. Blood samples for the measurement of tetanus antitoxin levels were taken 14 days after the immunization. Fifty-nine similarly immunized persons served as controls.

Plasma cortisol estimation. Plasma cortisol was determined by the method of Spencer-Peet, Daly & Smith (1965). The concentrations are expressed as μmol/l.

Leucocyte and lymphocyte counting. Erythrocytes were lysed with 2% acetic acid. The leucocytes were counted in a Bürker–Türk chamber. For the differential count, cell smears were stained with haematoxylin and eosin and 200 cells were counted in each smear by the same observer throughout the study.

Lymphocyte transformation test. Our routine method for lymphocyte stimulation with mitogens (Eskola *et al.*, 1975) and with PPD (Viljanen & Eskola, 1977) was used. In brief, a mixture of 25 μl of heparinized blood and 75 μl of RPMI 1640 (Grand Island Biological Co., Grand Island, New York) was placed into the wells of a sterile microtitre plate (IS-MRC-96, Linbro Chemical Co., New Haven, Conneticut). Thereafter, PHA (PHA M, Difco Laboratories, Detroit, Michigan), Con A (Pharmacia Fine Chemicals, Uppsala, Sweden) and PPD (Batch 288, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, Surrey) in 25 μl of RPMI 1640 were added into the desired wells. Control cultures received 25 μl of plain medium. The final concentrations of PHA and Con A were 100 μg/ml and those of PPD were 1·0 and 100 μg/ml. 18 hr before harvesting, 0·25 μCi of [5-^{125}I]iodo-2′-deoxyuridine ([^{125}I]UdR, sp. act. 90–110 mCi/mg, The Radiochemical Centre, Amersham) in 20 μl was added into the wells together with 5-fluoro-2′-deoxyuridine (Fluka, Buchs, Switzerland) to a final concentration of 10^{-6} M (Asantila & Toivanen, 1974). The mitogen-stimulated cultures were harvested with a multiple cell culture harvester (Skatron, Lierbyen, Norway) 72 hr and the PPD-stimulated cultures 96 hr after the beginning of the culture. The responses are expressed as counts per minute (ct/min).

Tetanus antitoxin estimation. IgG tetanus antitoxins were measured by ELISA according to Viljanen & Ruuskanen (to be published).

Statistics. The student's *t*-test was employed in the comparison of the means.

RESULTS

Effect of marathon running on plasma cortisol, leucocyte and lymphocyte counts and on lymphocyte transformation

In the first part of the study, the plasma cortisol, leucocyte and lymphocyte counts and lymphocyte transformation induced by PHA, Con A and PPD were measured in the samples taken from four runners 30 min before and after the marathon and 3 hr after the completion of the run. The results are summarized in Fig. 1. The mean level of plasma cortisol increased from 0·48 μmol/l to 1·08 μmol/l. In each case there was also a significant leucocytosis (7600 and 19,609 cells per mm^3, respectively) which was due to the granulocytosis. No significant change was found in the mean lymphocyte count (3196 and 2451 cells per mm^3, respectively). The lymphocyte transformation was clearly suppressed both in mitogen- and PPD-stimulated cultures. The most dramatic decreases were observed in a 50 year old man, in whom all the responses decreased to about 1/10 of the initial levels.

3 hr after the completion of the marathon, a slight decrease in the mean level of plasma cortisol was found. However, no clear recovery in the leucocyte counts and lymphocyte responses was observed, except in the lymphocyte transformation with 1 μg/ml of PPD.

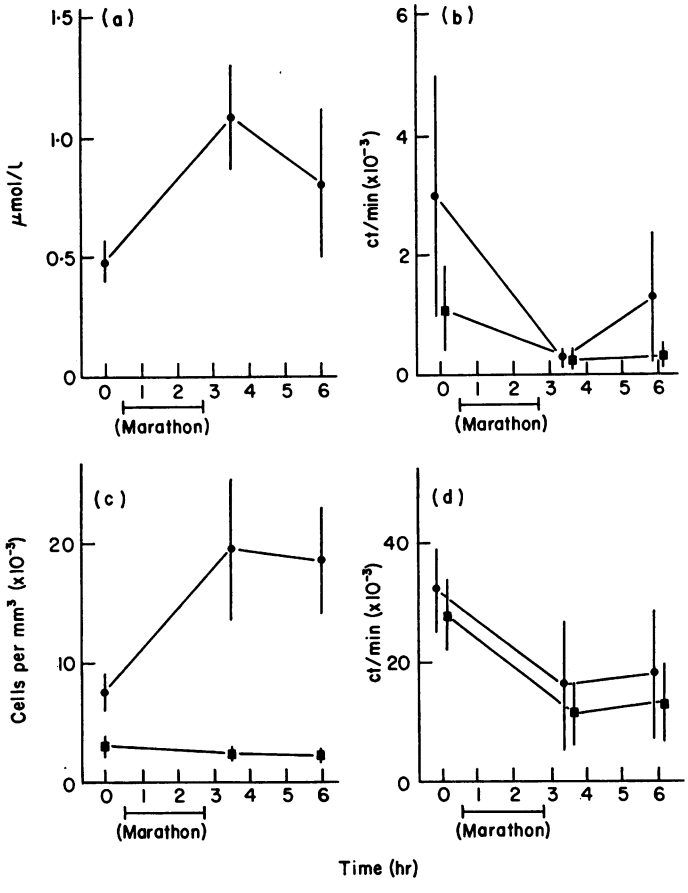

FIG. 1. Effect of marathon running on: (a) the level of plasma cortisol; (b) the lymphocyte transformation induced by PPD, 1 µg/ml (●) and 100 µg/ml (■); (c) the count of peripheral blood leucocytes (●) and lymphocytes (■); and (d) the lymphocyte transformation induced by PHA (●) and Con A (■). Mean values ± s.d. are presented.

Effect of 7 km of running on plasma cortisol, leucocyte and lymphocyte counts and on lymphocyte transformation

The same parameters as described above were measured in four runners 30 min before and after a 7 km of running and 3 hr after the run. The results are presented in Fig. 2. This exercise induced again a significant increase in the mean level of plasma cortisol immediately after the run (0·37 and 0·70 µmol/l, respectively). This change was not so profound as the increase after the marathon and the mean level returned to the initial level in 3 hr. In contrast to the findings after the marathon, the leucocytosis occurred only 3 hr after the run, without any changes in the lymphocyte counts. No significant suppression of the lymphocyte transformation could be demonstrated. Only a slight tendency towards a decreased response to PPD was observed 3 hr after the run.

Antibody formation and recovery of depression in lymphocyte transformation after marathon running

The suppression in the lymphocyte transformation found in the first part of the study led us to examine the recovery of this phenomenon in eight highly conditioned runners. In addition, the antibody formation capacity was measured in these runners by giving them a booster dose of tetanus toxoid. The immediate effect of the marathon on the plasma cortisol, leucocyte and lymphocyte counts and the lymphocyte transformation was quite similar to that found in the first part of the study (Fig. 3). The mean level of plasma cortisol increased from 0·41 µmol/l to 0·79 µmol/l. The mean leucocyte count showed a

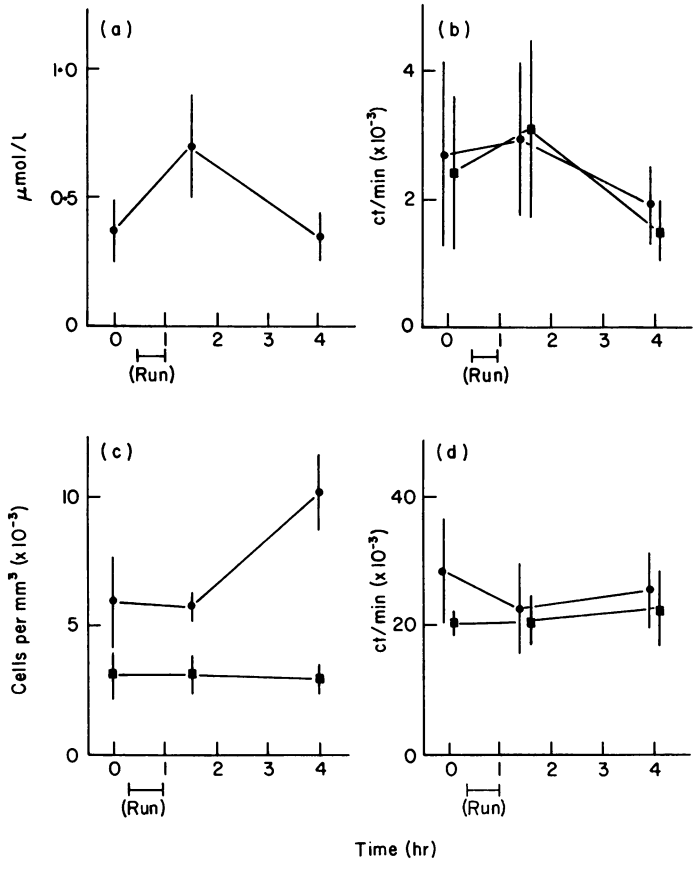

FIG. 2. Effect of 7 km running on: (a) the level of plasma cortisol; (b) the lymphocyte transformation induced by PPD, 1 μg/ml (●) and 100 μg/ml (■); (c) the count of peripheral blood leucocytes (●) and lymphocytes (■); and (d) the lymphocyte transformation induced by PHA (●) and Con A (■). Mean values ± s.d. are presented.

three-fold increase (5454 and 14,970 cells per mm^3, respectively) without any charge in the mean lymphocyte count (2558 and 2562 cells per mm^3, respectively). The reduction in the PHA and Con A responses was clearly demonstrable, but it was not so significant as in the first part of the study. The response of the peripheral blood lymphocytes to PPD stimulation decreased significantly to about 1/4 of the initial level.

Most of the parameters studied returned to the initial levels on the next day. Only the responses of the peripheral blood lymphocytes to both concentrations of PPD were a little lower than those before the running.

The antibody responses of individual runners and those of fifty-nine control persons are presented in Fig. 4. The initial values of the IgG tetanus antitoxins were mostly lower than 10% of the hyperimmunized standard plasma. The tetanus booster induced a clear-cut increase in the antitoxin concentration of the runners. The antibody levels of the runners ranged from 31·5% to 88·9% of the hyperimmunized standard plasma, exceeding the mean antitoxin level of the control persons.

DISCUSSION

Sport causes stress of different degrees, depending on the type and duration of the performance. It is mainly physical in character, but competitive events are also associated with psychic tension. Competitive marathon running can be considered as maximal physical stress, but it contains also components of

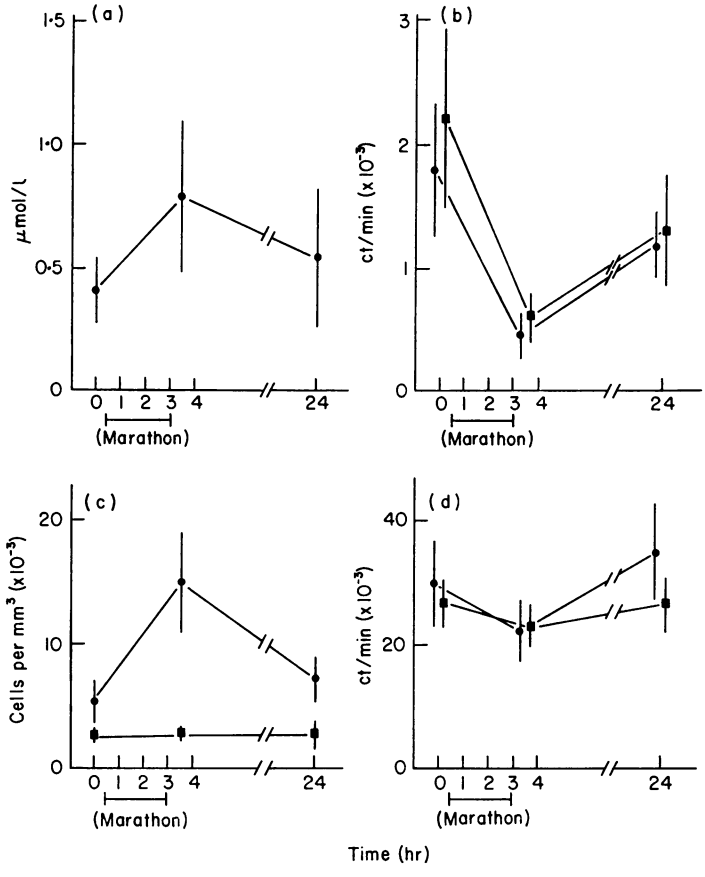

FIG. 3. Recovery of: (a) plasma cortisol; (b) lymphocyte transformation induced by PPD, 1 μg/ml (●) and 100 μg/ml (■), (c) peripheral blood leucocytes (●) and lymphocytes (■); and (d) of lymphocyte transformation induced by PHA (●) and Con A (■) after marathon running. Mean values ± s.d. are presented.

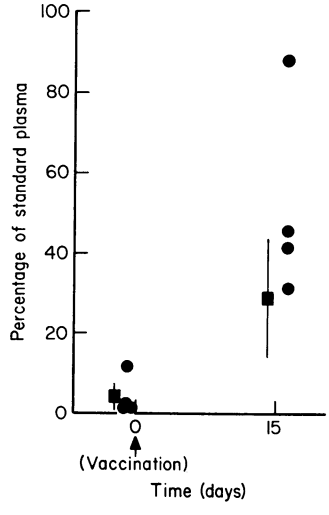

FIG. 4. Antibody response to tetanus toxoid vaccination in fifty-nine control persons (■) (mean value ± s.d.) and individual antibody responses of four marathon runners (●).

psychic stress. In this study we found a clear-cut depression in the transformation of blood lymphocytes after PHA, Con A and PPD stimulation after the marathon. The depression was simultaneous with granulocytosis and elevation of the plasma cortisol level. These two phenomena were also observed in the subjects running 7 km, without, however, any depression in the lymphocyte transformation. Accordingly, the impairment of lymphocyte functions in marathon runners can not be directly explained on the basis of cortisol effect. In the marathon running, the elevated cortisol levels were present for a longer period than in the shorter running. This might partly explain the difference in the results.

A very short (10–15 min) physical stress has been observed to increase the number of blood lymphocytes, particularly that of B cells (Steel *et al.*, 1974; Hedfors *et al.*, 1976; Yu *et al.*, 1977) and to decrease the response of isolated lymphocytes to mitogens and PPD, as well as the K-cell cytotoxicity (Hedfors *et al.*, 1976; Yu *et al.*, 1977). In this work we could not find any change in the lymphocyte count 30 min after a moderate (35 min) or a very heavy (2·5 hr) sport stress. There are at least two possible explanations for this discrepancy. Firstly, it is possible that a transient lymphocytosis has occurred in our runners, but during the continuation of the performance it has disappeared. Steel *et al.* (1974) have shown that the lymphocyte count returns to the pre-exercise level in 30 min after 10 min of vigorous exercise. This may explain our negative findings concerning the lymphocytosis because we collected blood samples just 30 min after the exercise. Secondly, the elevated plasma cortisol may prevent the exercise-induced lymphocytosis, as is found after administered corticosteroids (Yu *et al.*, 1977).

Hedfors *et al.* (1976) have shown that 15 min of bicycle ergometer test decreases significantly the reactivity of the peripheral blood lymphocytes to PHA, Con A, pokeweed mitogen and PPD stimulation. However, we could observe a significant decrease in the responsiveness of lymphocytes to mitogen and PPD stimulation only after 2·5 hr of running, not after 35 min of running. Our method for the measurement of lymphocyte transformation is different of that used by Hedfors and her colleagues; we utilized whole blood instead of isolated lymphocytes. It is not impossible that the changes in the responses of the peripheral blood lymphocytes can be detected sooner with isolated lymphocytes than with the whole blood. In addition, the presence of plasma factors having an influence on the reactivity of T lymphocytes cannot be excluded. The discrepancy may also be related, as said before, to the difference in the duration of the stress. This means that a decrease in the lymphocyte responses occurs after 15 min of stress and after that, due to the redistribution of lymphocytes induced by the plasma cortisol (Fauci, 1975), the lymphocyte responsiveness returns to the normal level. When the stress then continues, the lymphocyte transformation is again depressed.

Granulocytosis, which is known to follow corticosteroid administration (Bishop *et al.*, 1968) and which was also observed in our study, did not affect the lymphocyte transformation in our cultures using whole blood. There was no clear-cut depression of the lymphocyte responses after 35 min of stress, although a significant granulocytosis occurred.

Of particular interest are our findings that the *in vitro* response of the lymphocytes to PPD is more suppressed than that to mitogens. One explanation for this could be the reduction in the number of monocytes caused by the elevated plasma cortisol, as found after administered corticosteroids (Fauci & Dale, 1975; Yu *et al.*, 1974; Rinehart *et al.*, 1975). Fauci & Dale (1974) have shown that the addition of monocytes to cultures of cells depleted of monocytes by corticosteroid administration resulted in a partial reconstitutution of responses to antigens. They suggest that the responsiveness of lymphocytes to antigen stimulation is more dependent on monocytes than mitogens.

Regarding the antibody formation, our findings are suggestive for a relative B lymphocytosis. The responses of individual runners were higher than the mean antibody level of control persons. Monjan & Collector (1977) have recently shown that a short-term daily stress in mice induces a depression of B- and T-cell functions, while with longer exposures to this type of stress an enhancement occurs. In addition, Gisler *et al.* (1971) have demonstrated that stress, more than 6 hr prior to stimulation of isolated splenic lymphocytes with sheep red blood cells *in vitro*, leads to suppression of the immune response, whereas a time interval of only 15 min between the stress and sampling results in a normal or even enhanced immune response. On the other hand, hydrocortisone has been shown to enhance the epinephrine-induced stimulation of immunoglobulin synthesis *in vitro* (Sherman, Smith & Middleton,

1973). Catecholamines are also known to elevate during exercise (Banister & Griffiths, 1972; von Euler, 1974).

Finally, marathon running as a maximal psycho-physical stress causes many biochemical changes in the body, e.g. lactic acidosis. In addition, the muscles are exhausted. The impairment of lymphocyte functions may be one indicator of the general exhaustion of the body. As such a phenomenon, it may offer one further possibility for estimating the form of the competiting athletes. The depression of lymphocyte functions after an extreme sport stress may make them more susceptible for infections.

In summary, we have shown that the lymphocyte responsiveness is depressed only by a very heavy sport stress. The recovery of this hyporesponsiveness occurs in 24 hr. Humoral immune functions are not impaired by this type of stress, they even may be enhanced. Whether the changes in immune responses after sport stress have clinical significance remains to be clarified.

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